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Anesthesia and circulating tumor cells in primary breast cancer patients: a randomized controlled trial

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Abstract: BACKGROUND: The effect of anesthetic drugs on cancer outcomes remains unclear. This trial aimed to assess postoperative circulating tumor cell counts-an independent prognostic factor for breast cancer-to determine how anesthesia may indirectly affect prognosis. It was hypothesized that patients receiving sevoflurane would have higher postoperative tumor cell counts. METHODS: The parallel, randomized controlled trial was conducted in two centers in Switzerland. Patients aged 18 to 85 yr without metastases and scheduled for primary breast cancer surgery were eligible. The patients were randomly assigned to either sevoflurane or propofol anesthesia. The patients and outcome assessors were blinded. The primary outcome was circulating tumor cell counts over time, assessed at three time points postoperatively (0, 48, and 72 h) by the CellSearch assay. Secondary outcomes included maximal circulating tumor cells value, positivity (cutoff: at least 1 and at least 5 tumor cells/7.5 ml blood), and the association between natural killer cell activity and tumor cell counts. This trial was registered with ClinicalTrials.gov (NCT02005770). RESULTS: Between March 2014 and April 2018, 210 participants were enrolled, assigned to sevoflurane (n = 107) or propofol (n = 103) anesthesia, and eventually included in the analysis. Anesthesia type did not affect circulating tumor cell counts over time (median circulating tumor cell count [interquartile range]; for propofol: 1 [0 to 4] at 0 h, 1 [0 to 2] at 48 h, and 0 [0 to 1] at 72 h; and for sevoflurane: 1 [0 to 4] at 0 h, 0 [0 to 2] at 48 h, and 1 [0 to 2] at 72 h; rate ratio, 1.27 [95% CI, 0.95 to 1.71]; P = 0.103) or positivity. In one secondary analysis, administrating sevoflurane led to a significant increase in maximal tumor cell counts postoperatively. There was no association between natural killer cell activity and circulating tumor cell counts. CONCLUSIONS: In this randomized controlled trial investigating the effect of anesthesia on an independent prognostic factor for breast cancer, there was no difference between sevoflurane and propofol with respect to circulating tumor cell counts over time.

DOI: https://doi.org/10.1097/ALN.0000000000003409

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: https://doi.org/10.5167/uzh-191077
Journal Article
Accepted Version

Originally published at:
Hovaguimian, Frédérique; Braun, Julia; Z'graggen, Birgit Roth; Schläpfer, Martin; Dumrese, Claudia; Ewald, Christina; Dedes, Konstantin J; Fink, Daniel; Rölli, Urs; Seeberger, Manfred; Tausch, Christoph; Papassotiropoulos, Bärbel; Puhan, Milo A; Beck-Schimmer, Beatrice (2020). Anesthesia and circulating

tumor cells in primary breast cancer patients: a randomized controlled trial. Anesthesiology, 133(3):548-558.
DOI: https://doi.org/10.1097/ALN.0000000000003409

Anesthesia and circulating tumor cells in primary breast cancer patients: a randomized controlled trial

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Clinical trial number and registry URL: This trial was registered with ClinicalTrials.gov (NCT02005770 - https://clinicaltrials.gov/ct2/show/NCT02005770 - Principal investigator: Beatrice Beck-Schimmer, registration date: December 9, 2013).

Prior presentations: Abstract A2182, ASA Annual Meeting, October 24 2016, Chicago, Illinois (*Sevoflurane Supports Cell Differentiation of NK Cells Towards Mature Killing Cells in Patients Undergoing Breast Cancer Surgery*. Martin Schläpfer, Philipp Eugster, Tobias Piegeler, Beatrice Beck-Schimmer)

Acknowledgments: We would like to thank Sabine Kern (study nurse, Institute of Anesthesiology, University Hospital of Zurich, Zurich, Switzerland) for the coordination of this study, Anja Zabel (laboratory technician, Institute of Anesthesiology, University Hospital of Zurich, Zurich, Switzerland) for her help in conducting the laboratory experiments, and Dr. Sarah Haile (PhD, statistical consultant, Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Zurich, Switzerland) for her statistical support.

Word count:

Abstract: 295; Introduction: 454; Discussion: 976; Number of figures: 3; Number of tables: 3.

Supplementary material: 1 document consisting of 4 figures.

Abbreviated title: Anesthesia and circulating tumor cells

Summary statement: not applicable

Funding statement: This study was funded by the Swiss National Science Foundation, the *Stiftung zur Krebsbekämpfung* (Zurich, Switzerland), the Masikini Foundation (Triesen, Liechtenstein) and the Uniscientia Foundation (Vaduz, Liechtenstein). FH was funded by the University of Zurich ("Protected time for research" and "Filling the Gap" grants). The funding organizations had no role in the design and conduct of the study; in the collection, management, analysis, or interpretation of the data; or in the preparation, review, or approval of the manuscript. FH, JB, MP, and BBS had access to the raw data. The corresponding author had full access to all study data and takes final responsibility for the decision to submit for publication.

Conflicts of Interest:

MS has received travel support from Baxter (unrelated to the study). KJD received honoraria and consultancies from Roche, Novartis, AstraZeneca, Amgen, Tesaro, PharmaMar and Daiichi (unrelated to the study). CT received consultancies from Roche (not related to this work). BBS received a grant from Baxter AG (not related to this work). BBS was a participant of an Advisory Board Meeting of Baxter AG (not related to this topic). BBS received a speaker's fee from Abbvie, Switzerland (topic: "Pro/cons of volatile anesthetics") for a Grand Round talk in a Swiss Hospital. BBS has a patent 04/10/14 – 20140100278: Injectable formulation for treatment and protection of patients having an inflammatory reaction or an ischemia-reperfusion event; M. Urner, L.K. Limbach, I.K. Herrmann, W.J. Stark, B. Beck Schimmer, applied as Patent Cooperation Treaty (PCT) (internationally), July 2009.
The other authors declare no competing interests.

Contributors:

FH and BBS participated in study conception and design, data acquisition and interpretation, and critical revision of the manuscript. FH drafted the manuscript. JB and MP performed the statistical analyses, participated in data analysis and interpretation, and critical revision of the manuscript. BRZ, MS, CD, CE, KD, DF, UR, MS, CT and BP participated in data acquisition and critical revision of the manuscript. FH and BBS obtained funding. BBS supervised the study.

ABSTRACT

Background

The effect of anesthetic drugs on cancer outcomes remains unclear. We aimed to assess postoperative circulating tumor cells counts - an independent prognostic factor for breast cancer - to understand how anesthesia may indirectly affect prognosis. We hypothesized patients receiving sevoflurane would have higher postoperative tumor cells counts.

Methods

Parallel, randomized controlled trial conducted in two centers in Switzerland. Patients aged 18-85 years without metastases and scheduled for primary breast cancer surgery were eligible. Patients were randomly assigned to either sevoflurane or propofol anesthesia. Patients and outcome assessors were blinded. The primary outcome was circulating tumor cells counts over time, assessed at three time points postoperatively (0h, 48h, and 72h) by the CellSearch® assay. Secondary outcomes included maximal circulating tumor cells value, positivity (cut-off: ≥1 and ≥5 tumor cells/7.5ml blood), and the association between natural killer cell activity and tumor cells counts. This trial was registered with ClinicalTrials.gov (NCT02005770).

Results

Between March 2014 and April 2018, 210 participants were enrolled, assigned to sevoflurane (n=107) or propofol (n=103) anesthesia, and eventually included in the analysis. Anesthesia type did not affect circulating tumor cells counts over time (median CTC count [IQR] propofol: 1 [0-4] at 0h, 1 [0-2] at 48h, and 0 [0-1] at 72 h; sevoflurane: 1 [0-4] at 0h, 0 [0-2] at 48h and 1 [0-2] at 72h; rate ratio 1.27 [95%CI 0.95 – 1.71]; p=0.103) or positivity. In one secondary analysis, administrating sevoflurane led to a significant increase in maximal

tumor cells counts postoperatively. There was no association between natural killer cell activity and circulating tumor cells counts.

Conclusions

In this randomized controlled trial investigating the effect of anesthesia on an independent prognostic factor for breast cancer, there was no difference between sevoflurane or propofol with respect to circulating tumor cells counts over time.

INTRODUCTION

Breast cancer represents a major health issue: with more than 2 million new cases worldwide,[1] it is the most frequently diagnosed tumor and the leading cause of cancer deaths in women.[2] Despite primary treatment, between 6% of patients with localized tumors and 22% with nodal extension will face recurrence at 5 years.[3]

Most patients diagnosed with breast cancer undergo surgical treatment. There have been increasing concerns, however, that the perioperative period would promote tumor spreading, either directly (i.e. through tumor manipulation), or indirectly, since systemic inflammation may affect immune responses against tumor cells.[4] Evidence also suggests that anesthesia itself may contribute to distant spread: anesthetic drugs seem to interfere directly with tumor cell biology and to decrease natural killer cells cytotoxic activity, which plays a critical role in tumor cell destruction and tumor growth.[5,6]

Although these effects have been well documented in pre-clinical studies, their relevance in the clinical setting is still matter of debate: intravenous anesthesia has been suggested to result in better survival rates compared to inhalational anesthesia, but evidence was mostly driven by retrospective analyses, which are prone to important methodological limitations.[7-14] Conflicting findings also emerged from a few randomized controlled trials suggesting no effect on survival, but sample sizes were small, follow-up duration short, and multiple interventions were evaluated without an adequate control group.[15-17]

Large, well-designed randomized controlled trial are thus needed to clarify the effect of anesthetic drugs on cancer prognosis, but long follow-up periods often undermine the feasibility of such studies.

To overcome this issue, the use of biological markers as surrogates for prognosis may represent a valuable approach.[18] Among others, the presence of circulating tumor cells in

the peripheral blood has been identified as a particularly promising indicator.[19] Hematogenous dissemination seems to occur long before clinical or radiological signs of metastases develop,[20] which places circulating tumor cells at an ideal location in the causal pathway leading to distant disease.[21] There is also increasing evidence that circulating tumor cells are independently associated with a higher risk of disease recurrence and with reduced survival, both in non-metastatic and metastatic breast cancer.[22,23] In this respect, circulating tumor cells monitoring may represent a promising approach to better understand the effect of anesthesia on tumor behavior during the perioperative period. Therefore, we conducted a randomized controlled trial to evaluate the effect of intravenous (i.e. propofol) *versus* inhalational (i.e. sevoflurane) anesthesia on postoperative circulating tumor cells counts in primary breast cancer patients. A superiority design was used to test the hypothesis that postoperative circulating tumor cells counts would be higher in patients receiving sevoflurane. The association between immune cell responses (i.e. natural killer cell cytotoxic activity) and circulating tumor cells counts was assessed in an exploratory *in vitro* study nested within this trial.

METHODS

We used the CONSORT recommendations for the reporting of randomized trials.[24] This trial was approved by the local ethical committee (Zurich, Switzerland – Registration number: PB_2016-01791) and was registered with ClinicalTrials.gov (NCT02005770 - https://clinicaltrials.gov/ct2/show/NCT02005770 - Principal investigator: Beatrice Beck-Schimmer, registration date: December 9, 2013). The study protocol is available on ClinicalTrials.gov.

Trial Design and Participants

This was a parallel-group, randomized, controlled trial conducted at a university hospital (University Hospital of Zurich) and a private clinic (Hirslanden Group, Zurich) in Switzerland. Patients were considered eligible if they were aged 18 to 85 years, diagnosed with primary pre-invasive and invasive breast cancer without distant metastases (stage 0-III) and scheduled for surgery with or without axillary node dissection. Patients were excluded if they met one of the following criteria: pre-operative chemotherapy, possible immune impairment (i.e. auto-immune disease, HIV, other active cancer, American Society of Anesthesiologists (ASA) physical status IV-V), immunosuppressive or chronic opioids therapy, secondary surgery (e.g. for recurrence, reconstruction) or surgery performed under general anesthesia with concomitant regional anesthesia (i.e. epidural catheter, paravertebral blockade, wound infiltration with local anesthetics). Those with a known or suspected hypersensitivity or allergy to anesthetics were considered ineligible. Patients were approached on the day prior to surgery by research staff, who evaluated eligibility, obtained written informed consent, and enrolled the participants.

Randomization and blinding

Randomization was performed by research staff using a secure Internet-based system (www.randomiser.at) that stratified patients according to their ASA status and ensured concealment of random allocation. Patients were randomly assigned in a 1:1 ratio to either intravenous anesthesia (propofol group) or inhalational anesthesia (sevoflurane group). Patients remained blinded of their assignment group (standardized induction in both groups), as was the study personnel involved in circulating tumor cells measurements (i.e. outcome assessors did not have access to patient charts).

Procedures

Anesthesia induction was standardized in both groups using fentanyl (2-3 mcg/kg), thiopental (4-6 mg/kg) and rocuronium (0.6 mg/kg). Patients requiring a rapid sequence induction received rocuronium 0.9 mg/kg instead of 0.6 mg/kg. Further administration of fentanyl during surgery followed a standardized protocol (i.e. 2 mcg/kg, total amount: 5-10 mcg/kg). In the propofol group, anesthesia was maintained using a target-controlled infusion (TCI) device providing an intravenous propofol dose adjusted to keep bispectral (BIS) index values between 40 and 60; in the sevoflurane group, sevoflurane was provided to keep BIS index values between 40 and 60. Postoperative nausea and vomiting prophylaxis and perioperative analgesia followed standardized protocols that were applied until hospital discharge.

Outcome

The primary outcome was the number of circulating tumor cells assessed postoperatively by the CellSearch® assay (Menarini Silicon Biosystems Inc, Huntingdon Valley, PA, USA). Based on immunomagnetic separation, this detection technique uses a magnetic field to isolate ferrofluid-labelled tumor cells of epithelial origin, such as breast cancer cells.[25] This standardized procedure uses antibodies directed against a common molecular signature

displayed by circulating tumor cells in breast cancer patients (i.e."EpCAM+/CK+/DAPI+/CD45-" signature – EpCAM: epithelial cell adhesion molecule; CK: cytokeratin; DAPI: 4',6-diamidino-2-phenylindole). After staining of the isolated cells, circulating tumor cells identification was confirmed by two independent, specifically trained laboratory technicians that were masked to treatment assignment. Identification of circulating tumor cells followed a predefined set of criteria (i.e. morphological features, compatible staining pattern).

Peripheral blood was collected at four different time points, i.e. before the induction of anesthesia (baseline), after surgery but prior to extubation (0h), at day-2 (48h) and day-3 (72h) postoperatively. The last measurement (72h) was initially planned on day-4, but was rescheduled to day-3 in January 2016 to avoid data loss due to early hospital discharge. This was the only change made to the original trial design.

Secondary outcomes were defined as: the maximal circulating tumor cells count value at any time point after surgery (0h, 48h and 72h); circulating tumor cells counts as a binary outcome (using 2 different cut-off values, i.e. ≥1 and≥5 circulating tumor cells/7.5ml blood); and the association between natural killer cell activity and circulating tumor cells counts (see also section "additional analyses"). Initially, only a cut-off value of ≥5 circulating tumor cells/7.5ml blood was considered. We added the ≥1 threshold at the time of analysis, since evidence suggested that values as low as 1 circulating tumor cell/7.5ml blood were associated with poorer prognosis in primary breast cancer patients.[22] No other changes were made to primary/secondary outcomes definitions over the study period.

Statistical analyses

Sample size calculation was performed using a method accounting for repeated measurements of count data over time.[26] Since evidence on the effect of intravenous or

inhalational anesthesia on circulating tumor cells counts was nonexistent, we adopted a conservative approach and assumed that the expected effect size (Cohen's d) between groups would be small (0.3). Thus, assuming a within-subject correlation of circulating tumor cells counts over time of 0.4 and a dropout rate of 10%, we estimated that a total of 232 patients would be required (209 patients without dropout) to detect a difference between groups corresponding to an effect size of 0.3, with a power of 80%, at a significance level of 5% (two-sided). Because the dropout rate was particularly low, the trial ended after enrolling 217 patients.

All analyses were based on intention-to-treat. Continuous data were expressed as means and standard deviations or as medians and interquartile ranges if distributions were skewed. The primary analysis used a mixed Poisson model with random intercept per patient to account for repeated measurements over time and thus correlated observations within-subjects. We opted for this approach, since the Poisson model is appropriate for count data (primary outcome of CTC counts). The results of the Poisson models are presented as rate ratios, denoting the comparison of circulating tumor cells counts between the two groups. To avoid assuming a linear development of circulating tumor cells over time, time was alternatively included as a factor variable in our model. We also explored the effect of anesthetics on the maximal circulating tumor cells count value at any time point after surgery in additional Poisson models (0h, 48h and 72h).

Since circulating tumor cells detection is usually reported as a binary outcome (i.e. positive *versus* negative endpoint using a cut-off value of ≥1 or ≥5 circulating tumor cells/7.5ml blood), circulating tumor cells count data were dichotomized and further assessed using a mixed logistic regression model with random intercept per patient.

Finally, models were adjusted to account for tumor-related and perioperative factors presumed to affect circulating tumor cells counts (i.e. tumor size, tumor type, overall opioids consumption – all preplanned).

All statistical analyses were conducted in R, version 3.6.1. Two-sided tests were performed and a level of significance of 0.05 was used.

Additional analyses

Because of the interplay between natural killer cell cytotoxic activity and tumor growth, we also assessed natural killer cell activity (i.e. apoptosis rate induced in tumor cells) in a preplanned, exploratory, *in vitro* study nested within this trial. Natural killer cell-induced apoptosis was evaluated in a subgroup of patients randomly selected from the study dataset. For each patient, natural killer cell activity was assessed at a single, pre-defined time point, i.e. when circulating tumor cells counts reached their maximal value. The association between natural killer cell-induced apoptosis rate and circulating tumor cells count was then assessed using linear regression analysis.

Natural killer cell-induced apoptosis rate and necrosis rate were determined *in vitro* by measuring target cell killing of the K562 tumor cell line (human chronic myelogenous leukemia, ATCC, CCL-243).[27,28] Patients blood samples were collected in EDTA-coated vials. Buffy coats (Blutspende Zürich, Schlieren, Switzerland) were used as controls. Peripheral blood mononuclear cells (PBMC) of both patient samples and buffy coats were isolated by Ficoll-Hypaque density gradient centrifugation and stored in liquid nitrogen. For determination of natural killer cell activity, PBMC were thawed and co-incubated with K562 for 24 h at 37°C/5% CO_2 in 10% human serum/RPMI. An effector (natural killer cells) to target cell (K562 cells) ratio of 1:1 was used. All cells were then washed in phosphate-buffered saline (PBS) and stained in 2% bovine serum albumin in PBS for 25 min at 4°C using

the following panel: CD3-APC (lymphocyte staining, Biolegend, London, UK), dilution of 1:100; CD 56-PE (natural killer cells staining, Biolegend, London, UK), dilution 1:100; CD16-FITC (FcγRIIIA staining, which is essential for cellular cytotoxicity, expressed on the surface of a subset of monocytes, Biolegend, London, UK), dilution 1:200. After a washing step in Annexin-V binding buffer, cells were simultaneously stained with Annexin-PerCPCy5.5 for staining of apoptotic cells (Biolegend, London, UK) at a dilution of 1:20, and Zombie-NIR for staining of necrotic cells (Biolegend, London, UK) at a dilution of 1:500.

Zombie-NIR-stained K562 boiled for 5 min at 80°C or Annexin-V-stained apoptotic K562, treated for 24h with 10 mM benzamide, were used as positive controls for cytotoxicity. Unstained K562, unstained patient PBMC and unstained PBMC from buffy coats served as negative controls. Cell analysis was performed using the spectral analyzer SP6800 (Sony Biotechnology Surrey, UK).[29]

RESULTS

Between March 10, 2014 and April 10, 2018, 586 patients were assessed for eligibility (figure 1). Of 217 enrolled participants, seven patients withdrew consent after randomization. We eventually included 210 patients in the intention-to-treat analysis (sevoflurane group: n=107, propofol group: n=103).

Baseline characteristics are presented in table 1. Demographic and clinical data were balanced between treatment groups. Most participants were middle aged, modestly morbid patients with an early-stage tumor. Baseline circulating tumor cells counts and positivity (using a cut-off value of ≥1 and ≥5 circulating tumor cells/7.5ml blood) were similar in both allocation groups. Table 2 depicts the intra- and postoperative characteristics, which were well-balanced between groups.

The evolution of circulating tumor cells counts over time is illustrated in Figure 2, table 3, and Supplemental Digital Content Figure 1 (which depicts predicted tumor cell counts using the estimates from the Poisson model, including a linear time variable and baseline circulating tumor cell counts). Administrating sevoflurane *versus* propofol did not affect the primary outcome of circulating tumor cells counts over time (rate ratio 1.27 [95%CI 0.95 - 1.71]; p = 0.103). This was the case, regardless of whether time was considered as a linear or a factor variable, or if an interaction term between time and anesthesia was introduced. However, when we explored the effect of anesthetics on the maximal circulating tumor cells value at any time point after surgery, administrating inhalational anesthesia (i.e. sevoflurane) led to a significant increase in maximal circulating tumor cells counts postoperatively (sevoflurane *versus* propofol: rate ratio 1.36 [95%CI 1.18 - 1.56]; p < 0.0001, i.e. the maximum number of circulating tumor cells increased by a factor of 1.36 (or 36 %) when sevoflurane was used compared to propofol).

When circulating tumor cells were analyzed as a binary outcome over time, the type of anesthesia did not have any effect on circulating tumor cells positivity, regardless of the cut-off value considered (cut-off value ≥1 circulating tumor cells/7.5ml blood: sevoflurane *versus* propofol, odds ratio 1.21 [95%CI 0.84 - 1.74]; p = 0.309; cut-off value ≥5 circulating tumor cells/7.5ml blood: sevoflurane *versus* propofol, odds ratio 1.59 [95%CI 0.86 - 3.01]; p = 0.139). Similar results were obtained when time was considered as a factor variable, and there was no evidence for an interaction between treatment and time.

We performed predefined analyses to explore whether tumor-related and perioperative factors modified the effect of anesthetics on circulating tumor cells counts. Models adjusted for tumor type (DCIS, luminal A, luminal B, Triple negative, HER2 positive, other) and tumor size (Tis, T1, T2, T3, T4) did not reveal any relevant effect modification on circulating tumor cells counts over time or positivity (regardless of the cut-off value considered). Similarly, adjusting for opioid consumption did not yield any effect modification. In the exploratory models, however, the effect of inhalational anesthesia on maximal postoperative circulating tumor cells values remained robust (sevoflurane *versus* propofol, rate ratio 1.26 [95%CI 1.09 - 1.47], p = 0.002, adjustment for tumor type, size and opioid consumption).

Exploratory *in vitro* analyses were conducted in a subgroup of 60 patients randomly selected from the study dataset (30 in the sevoflurane group, 30 in the propofol group). Similar natural killer cell-induced apoptosis rates were found in both treatment groups (mean apoptosis rate, sevoflurane group: 34.7%; propofol group: 35.7%). Overall, necrosis rate of K562 tumor cells was below 1%. Linear regression yielded no evidence for an association between apoptosis rates and maximal circulating tumor cells counts (regression coefficient -0.077, 95%CI -0.33 to 0.17 – figure 3). This was the case, regardless of treatment group

assignment, or whether an interaction term between anesthesia type and natural killer cell activity was introduced.

DISCUSSION

In this randomized controlled trial including 210 participants undergoing surgery for primary breast cancer, the type of anesthesia did not seem to affect circulating tumor cells counts over time or circulating tumor cells positivity. In one secondary analysis, there was a 36% increase in the maximal number of postoperative circulating tumor cells in patients receiving inhalational anesthesia. Additional *in vitro* analyses in a random selection of 60 patients did not reveal any evidence for an association between natural killer cell-induced apoptosis rates and maximal circulating tumor cells counts.

This trial investigated the effect of anesthesia on perioperative circulating tumor cells counts, an independent prognostic factor for breast cancer. In contrast to previously published randomized trials,[15-17] our study was larger, had an adequate control group, and the issue of long follow-up periods was mitigated by using a prognostic factor.

In our trial, circulating tumor cells counts at baseline were higher than those reported in previous studies. Several reasons may account for this discrepancy: first, all of our patients underwent sentinel lymph node localization 18 to 24h before baseline circulating tumor cells assessment and we cannot formally exclude that an injection in the vicinity of the tumor would not lead to any circulating tumor cells release. Second, approximately 30% of our patients had wire-guided localization of the tumor, which implies direct manipulation of the tumor short before circulating tumor cells assessment.

Since the identification of circulating tumor cells with the CellSearch® assay may imply some degree of subjectivity (i.e. images of potential tumor cells candidates are displayed to trained laboratory technicians and assessed following pre-defined criteria), we verified all samples with ≥5 tumor cells/7.5ml blood using the automated software ACCEPT (Supplemental Digital Content Figure 2, illustrating the flow chart of the validation

analysis).[30] Overall, the comparison showed a good correlation (Supplemental Digital Content Figure 3, illustrating the correlation between these 2 methods). Compared to the ACCEPT software, there was an overestimation of circulating tumor cells counts by 1.66 units with human assessment (Supplemental Digital Content Figure 4, illustrating the agreement between these 2 methods). However, in this validation analysis, only samples with high tumor cells counts were considered. This may bias the results towards an overestimation of the difference in means. In other words, if all samples, i.e. including those with 0 to 4 tumor cells/7.5ml blood had also been included, the difference in means of 1.66 units would have likely been smaller. Secondly, the overestimation of 1.66 units was non-differential, i.e. applied to both groups, regardless of treatment assignment.

Apart from one secondary analysis, our findings contrast with numerous previously published studies suggesting better outcomes with the use of intravenous anesthesia. The potential reasons for this disparity are two-fold. Firstly, clinical studies reporting on cancer outcomes were based on retrospective data analyses,[7-14] which are prone to bias and confounding. Secondly, evidence of a protective effect associated to propofol was partly driven by *in vitro* studies,[31-35] which may not reflect the delicate interplay between immune and tumor cells observed *in vivo*. Our findings, however, are consistent with a recently published, large, randomized controlled trial addressing the effect of regional *versus* general anesthesia on breast cancer recurrence.[36] Although this trial was not specifically designed to compare inhalational with intravenous anesthesia, most patients allocated to general anesthesia received sevoflurane, whilst those allocated to regional anesthesia received propofol. In line with our study, this trial failed to show any difference in cancer outcomes.

Our results, however, need to be interpreted with caution. Firstly, we assumed circulating tumor cells counts would be an appropriate prognostic factor to measure the impact of

anesthesia on the risk of tumor recurrence, but we did not perform a long-term outcome analysis to confirm this assumption. Although many oncological markers seem to be ideally placed in the causal pathway leading to distant disease, several other factors will eventually be needed to result in metastatic spread and uncertainty regarding the ability of these prognostic factors to predict "hard endpoints" must be acknowledged.[37] A second concern is that the exact meaning of circulating tumor cells changes in the perioperative period remains unclear. In studies investigating the predictive validity of circulating tumor cells changes in primary and metastatic breast cancer, patients converting from "positive" to "negative" status were found to have longer progression-free survival and overall survival than those with a persisting "positive" status.[23,38-42] However, circulating tumor cells detection was performed over many weeks or months and there is no firm evidence that these findings also apply to the immediate and rather short perioperative period. Other limitations are inherent to the CellSearch assay itself. Whilst the pattern "EpCAM+/CK+/DAPI+/CD45-" is a widely accepted molecular circulating tumor cells signature, other combinations may also occur: it has been argued, for instance, that 7.8% to 10.3% of breast cancers might lack EpCAM expression.[43,44] Further skepticism has been partly related to the fact that for a given tumor, a variety of circulating tumor cells phenotypes seems to exist.[45] Thus, in some patients included in our study, the ability to detect circulating tumor cells might have been hampered by the technique used. Finally, the *in vitro* analysis was performed in a sample of 60 patients only, thereby limiting our ability to fully assess the association between natural killer cell-induced apoptosis rates and circulating tumor cells counts. The risk of other sources of bias (such as selection, performance, attrition and detection bias) was deemed low.

In this randomized controlled trial, we investigated the effect of anesthesia on an independent prognostic factor in primary breast cancer patients. There was no difference in circulating tumor cells counts over time or circulating tumor cells positivity between patients receiving sevoflurane or propofol. One secondary analysis suggested a favorable effect of propofol on maximal postoperative circulating tumor cells values. Trials collecting long-term outcomes (NCT02786329, NCT03034096, NCT01975064, and NCT02660411) will bring further evidence regarding the possible effects of anesthesia during cancer surgery.

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FIGURE LEGENDS

Fig. 1. Flow diagram

Fig. 2. Evolution of circulating tumor cells counts over time.

Fig. 3. Scatter plot of natural killer cell activity and maximal circulating tumor cells counts, by treatment.

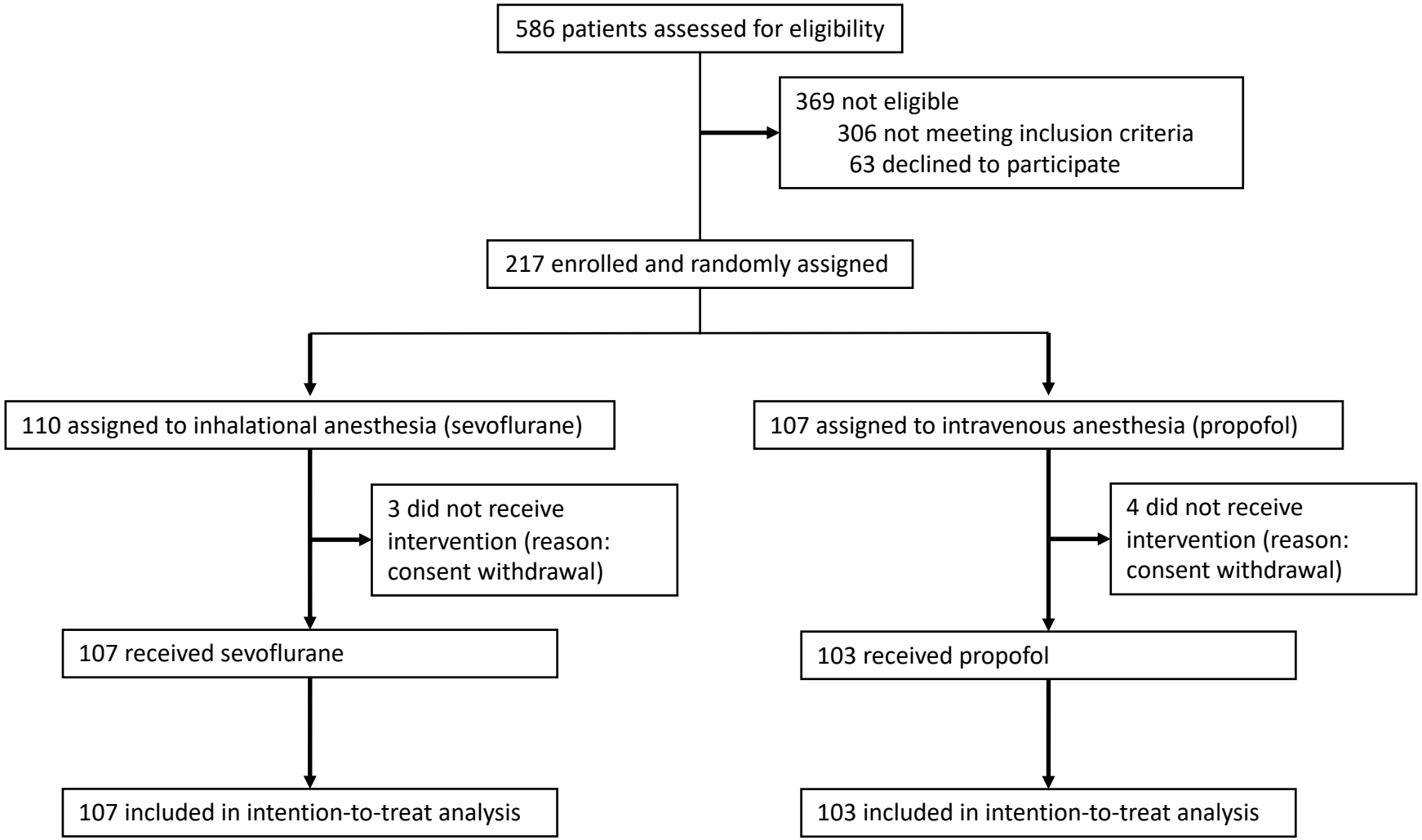

Fig. 1. Flow diagram

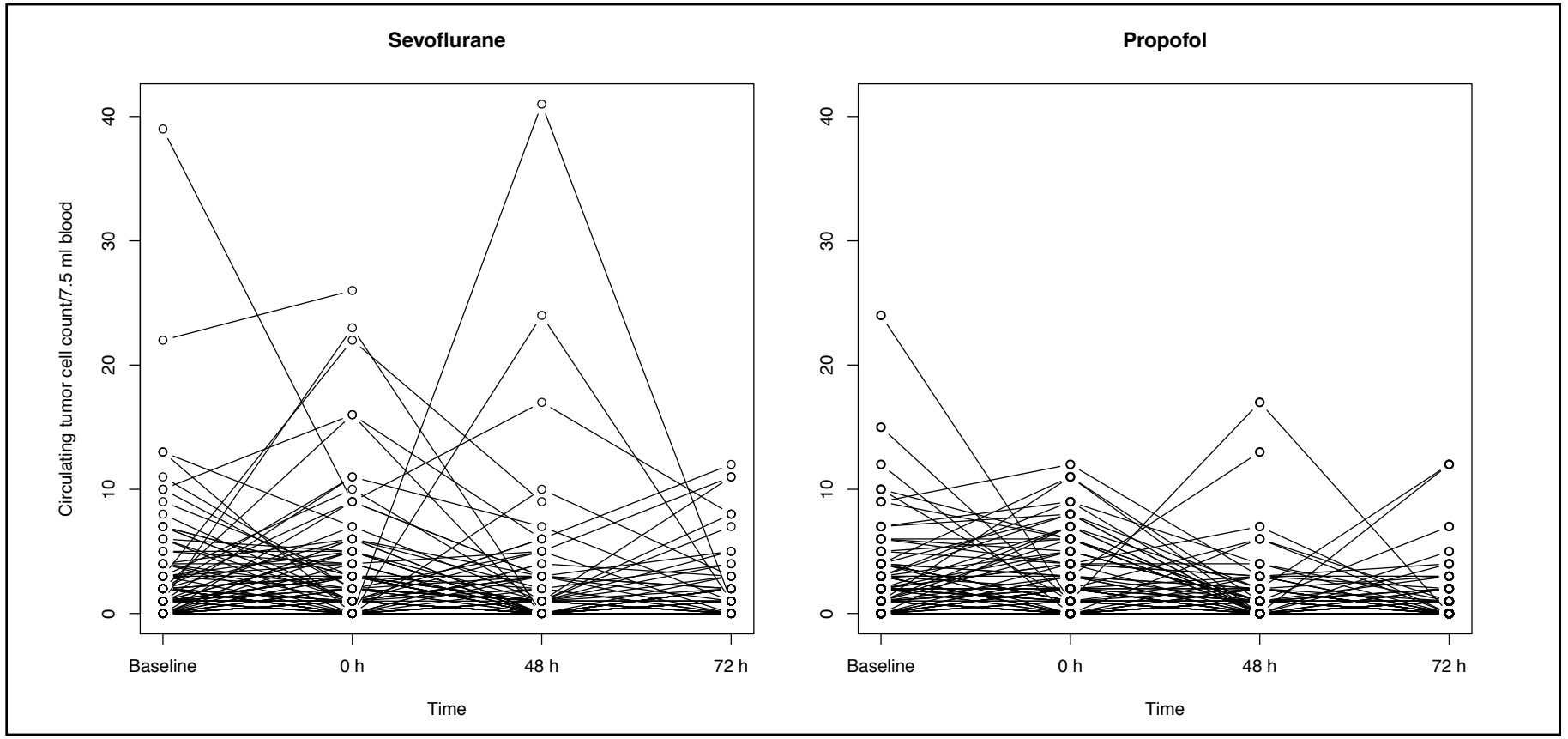

Fig. 2. Evolution of circulating tumor cells counts over time.

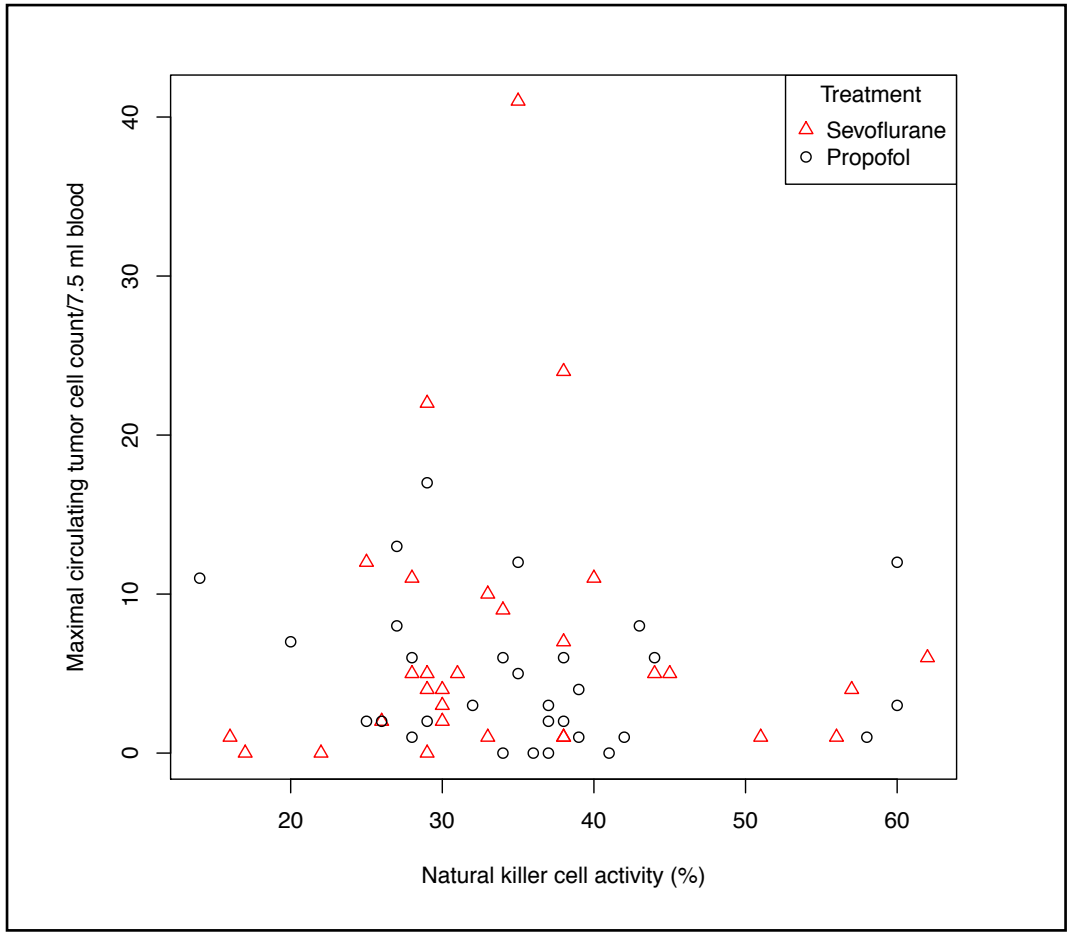

Fig. 3. Scatter plot of natural killer cell activity and maximal circulating tumor cells count, by treatment.

Table 1. Baseline characteristics		
	Sevoflurane (n=107)	**Propofol (n=103)**
Age, years	59 (13)	59 (12)
Body mass index, kilogram/square meter	26.7 (6.1)	26.2 (5.6)
ASA class		
I	29 (27.1)	25 (24.3)
II	73 (68.2)	73 (70.9)
III	5 (4.7)	5 (4.8)
Tumor size		
Carcinoma in situ	9 (8.4)	5 (4.9)
T1, < 2cm	55 (51.4)	55 (53.4)
T2, 2-5 cm	38 (35.5)	33 (32.0)
T3, > 5cm	3 (2.8)	7 (6.9)
T4, any size, growing into the chest wall or skin	1 (0.9)	2 (1.9)
Not reported	1 (0.9)	1 (1.0)
Pathological nodal status		
N0, node-negative	65 (60.7)	62 (60.2)
N1, 1-3 lymph nodes	29 (27.1)	22 (21.4)
N2, 4-9 lymph nodes	4 (3.7)	6 (5.8)
N3, >10 lymph nodes or infra-/supraclavicular	1 (0.9)	2 (1.9)
Not reported	8 (7.5)	11 (10.7)
Receptors		
Estrogen receptor - /Progesterone receptor -	11 (10.3)	12 (11.7)
Estrogen receptor +/Progesterone receptor -	9 (8.4)	7 (6.8)
Estrogen receptor -/Progesterone receptor +	0 (0)	0 (0)
Estrogen receptor +/Progesterone receptor +	85 (79.4)	76 (73.8)
Human epidermal growth factor 2 receptor + (immunohistochemistry score 3+)	7 (6.5)	14 (13.6)
Data are mean (SD) or n (%), unless otherwise specified. ASA: American Society of Anesthesiologists.		

Table 1. Baseline characteristics (continued)		
	Sevoflurane (n=107)	**Propofol (n=103)**
Tumor type*		
Ductal carcinoma in situ	8 (7.5)	4 (3.9)
Luminal A	58 (54.2)	58 (56.3)
Luminal B	19 (17.8)	12 (11.7)
Triple negative	5 (4.7)	6 (5.8)
Human epidermal growth factor 2 receptor status positive (fluorescence in situ hybridization)	9 (8.4)	16 (15.5)
Other	2 (1.9)	3 (2.9)
Not reported	6 (5.6)	4 (3.9)
Surgery type		
Lumpectomy with lymph node resection	76 (71.0)	70 (68.0)
Lumpectomy without lymph node resection	6 (5.6)	5 (4.8)
Quadrantectomy with lymph node resection	5 (4.7)	3 (2.9)
Quadrantectomy without lymph node resection	0 (0.0)	1 (1.0)
Modified radical mastectomy	3 (2.8)	6 (5.8)
Radical mastectomy	13 (12.2)	14 (13.6)
Other	4 (3.7)	4 (3.9)
Circulating tumor cells		
Number, median [interquartile range]	1 [0 - 3]	1 [0 - 3]
Circulating tumor cells positivity, cut-off value: ≥1 cell/7.5ml blood	73 (69.5)	68 (68.7)
Circulating tumor cells positivity, cut-off value: ≥5 cell/7.5ml blood	18 (17.1)	15 (15.2)
Data are mean (SD) or n (%), unless otherwise specified. *Based on guidelines from the European Group on Tumor Markers.		

Table 2. Intra- and postoperative characteristics		
	Sevoflurane (n=107)	Propofol (n=103)
Duration of anesthesia, min	163 (78)	167 (50)
BIS value, median [IQR]	43 [40 - 48]	36 [30 - 40]
Core temperature, degrees Celsius	36.2 (0.5)	36.2 (0.4)
Fentanyl, mg, median [IQR]	0.4 [0.4 - 0.5]	0.5 [0.4 - 0.6]
Morphine PACU intravenous, mg, median [IQR]	0.0 [0.0 - 4.0]	0.0 [0.0 - 2.5]
NSAID administration		
Intraoperative	13 (12.2)	9 (8.7)
Postoperative	95 (88.8)	92 (89.3)
Intraoperative radiotherapy	70 (65.4)	71 (68.9)
Data are mean (SD) or n (%), unless otherwise specified. BIS: bispectral index; NSAID: non-steroidal antiinflammatory drugs; PACU: postanesthesia care unit.		

Table 3. Perioperative circulating tumor cells counts						
Time point	Allocation group	Number of patients	Min	Median	IQR	Max
Baseline	Sevoflurane	105	0	1	[0 - 3]	39
	Propofol	99	0	1	[0 - 3]	24
0 hour	Sevoflurane	107	0	1	[0 - 4]	26
	Propofol	100	0	1	[0 - 4]	12
48 hours	Sevoflurane	100	0	0	[0 - 2]	41
	Propofol	94	0	1	[0 - 2]	17
72 hours	Sevoflurane	81	0	1	[0 - 2]	12
	Propofol	79	0	0	[0 - 1]	12
IQR: interquartile range.						

Supplemental Digital Content

Anesthesia and circulating tumor cells in primary breast cancer patients: a randomized controlled trial

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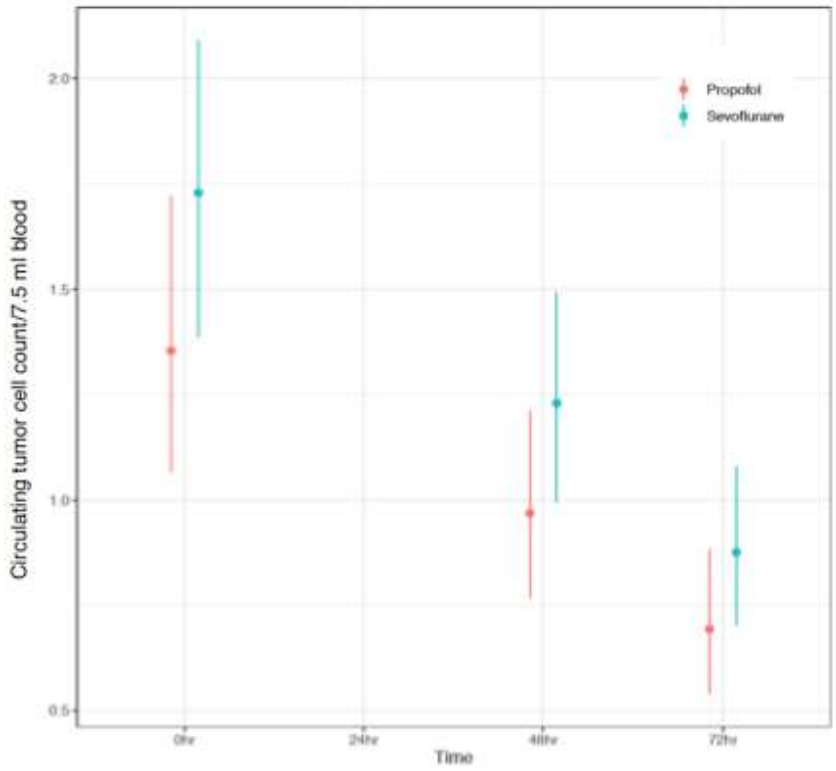
Figure 1. Predicted mean circulating tumor cell counts using primary Poisson model estimates



Estimates from the Poisson model including a linear time variable and baseline circulating tumor cell counts were used to predict the mean response for each of the two treatment groups and time points including 95% bootstrap confidence intervals.

Figure 2. Flow chart of the validation analysis

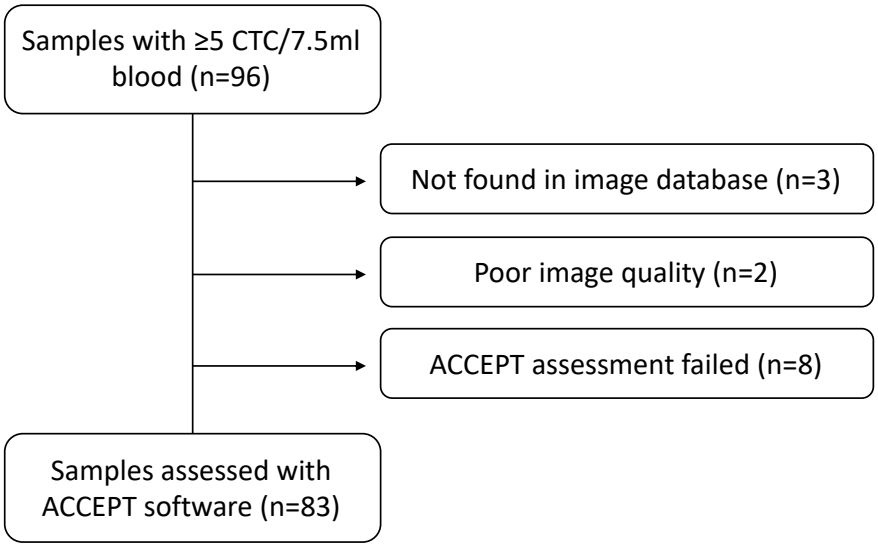


Figure 3. Scatterplot of original versus ACCEPT measurements

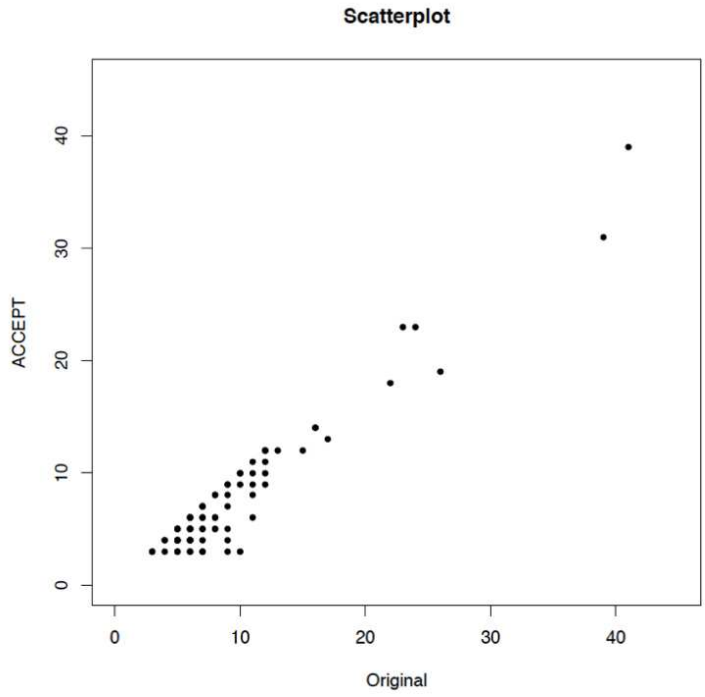


Figure 4. Bland-Altman plot of original versus ACCEPT measurements